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(54) Title: PROTECTIVE PROSTAGLANDINS FOR USE IN CONJUNCTION WITH CHEMOTHERAPEUTIC AGENTS

## (57) Abstract

Prostaglandins of the PGE type can be administered in a therapeutically effective amount to protect tissue from injury resulting from chemotherapeutic agents by administering the PGE-type prostaglandin prior to administration of the chemotherapeutic agent.

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Protective Prostaglandins for use in  
Conjunction with Chemotherapeutic Agents

Background of the Invention

This application is a continuation-in-part of U.S. Application Serial No. 07/886,270, filed May 20, 1992.

5       The present invention provides a method for protecting a variety of normal cells and tissues from injury produced by cytotoxic agents administered for the purpose of cancer therapy (chemotherapeutic agents). Specifically, it has been found that  
10       prostaglandins can protect normal tissues, such as hair and hair follicles and the hematopoietic system including protection of white blood cell (WBC) precursors in the bone marrow, from injury resulting from the administration of chemotherapeutic agents.  
15       Other tissues which can be protected include but are not limited to the following: skin; the gastrointestinal tract including stomach (gastric tissue), small intestine, colon and colo-rectal tissue; the esophagus, the mouth, including the oral mucosa;  
20       kidney, vagina, bladder, nasal passages, and eye. Prevention of tissue injury may be beneficial in reducing the morbidity of cancer chemotherapy and/or allowing for a higher and more curative dose regimen of chemotherapy to be delivered to cancer patients without  
25       damage or with minimized damage to the normal cells of such tissues.

      The present invention relates to a method for protecting a variety of types of normal cells and tissues in the body from injury produced by chemical  
30       agents commonly used in the chemotherapy of human cancer. It has been found that the systemic administration or the topical application of misoprostol, [also known as, ( $\pm$ )(16RS)-15-deoxy-16-hydroxy-16-methyl prostaglandin E<sub>1</sub>, methyl ester and

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commercially available as Cytotec® from G. D. Searle & Co.] or the systemic administration of (±)methyl 7-[3α-hydroxy-2β-(4R-hydroxy-4-methyl-1E,5E,7E-nonatrienyl)-5-oxo-1α-cyclopentyl]-4Z-heptenoate, or 16,16-dimethyl  
5 prostaglandin E<sub>2</sub> or PGE<sub>1</sub> prior to the administration of cytotoxic chemotherapeutic agents serves to protect animals from death and/or protects different cell types in normal tissues from injury or damage caused by chemotherapeutic agents.

10 People diagnosed as having cancer are frequently treated with single or multiple cytotoxic chemotherapeutic agents (cytotoxic agents) to kill cancer cells at the primary tumor site or at distant sites to where cancer has metastasized. Chemotherapy  
15 treatment is given either in a single or in several large doses or, more commonly, it is given in small doses 1 to 4 times a day over variable times from weeks to months. There is a large number of cytotoxic agents used to treat cancer and the mechanisms of the  
20 cytotoxic effects of each agent is frequently not known or only partially known. Irrespective of the mechanism, useful chemotherapeutic agents are known to injure and kill cells of both tumors and normal tissues. The successful use of chemotherapeutic agents  
25 to treat cancer depends upon the differential killing effect of the agent on cancer cells compared to effects on critical normal tissues. The effects of chemotherapeutic agents on normal tissues are referred to as side-effects of cancer treatment. The immediate  
30 side effects (minutes to a few hours) of chemotherapy may include dizziness, nausea, vomiting, and diarrhea. These side effects are uncomfortable but, in themselves, are not life-threatening. Cell killing or damage within normal tissues that occurs from days to  
35 weeks during a course of chemotherapy may result in uncomfortable and/or life threatening side effects. Among these effects are hair loss, hearing loss,

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sterility, damage to the mucosal epithelium of the gastrointestinal tract, damage to the oral mucosa, esophagus, small and large intestines, kidney damage, skin damage, cardiac damage, killing and suppression of the white blood cells which can lead to infection, and killing of hematopoietic blood forming cells. Many of these side effects are related to tissues and organ systems that have a high number of dividing cells (proliferative cells). Some of these side effects are non-life threatening; however, a reduction or prevention of these effects could have a beneficial effect on cancer patients or make it possible to administer a higher dose of the chemotherapeutic agent while minimizing damage or death of cells in normal tissue.

Hair loss (alopecia) caused by chemotherapeutic agents is an example of a non-life-threatening side effect which can be prevented or minimized. Alopecia is typically viewed by both male and female patients as a sign of rapid progress of their disease and not as a side effect of the chemotherapy treatment. Alopecia frequently produces anxiety and a loss of self esteem in cancer patients. A reduction or prevention of non-life threatening side effects of chemotherapy such as alopecia could increase a cancer patient's quality of life and outlook during treatment.

Other side effects of chemotherapy treatment are life threatening and thus limit the doses of chemotherapeutic agents that can safely be delivered to a cancer patient undergoing treatment. A reduction or prevention of dose-limiting, life-threatening side effects of chemotherapy could reduce the risk of injury or death of normal cells in the patient and possibly allow the administration of a larger dose of chemotherapeutic agents, thereby increasing the longevity and the number of survivors following chemotherapy.

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Prevention or protection from the side effects of chemotherapy would be a great benefit to cancer patients. Previous efforts to reduce these side effects have been many, varied and largely  
5 unsuccessful. For non-life-threatening side effects, little effort has been expended. Attempts to prevent hair loss, for example, have included use of a cold cap or tourniquets to reduce drug concentrations in the scalp. Patient compliance is poor and these methods  
10 are infrequently used. More recently, a compound called ImuVert (made by Cell Technology, Inc.) has been reported to reduce hair loss in rats given anti-cancer agents, but the flu-like side effects make it impractical (See Hussein, A.M.; Jimenez, J.J.; McCall,  
15 C.A.; Yunis, A.A., Protection from Chemotherapy Induced Alopecia in a Rat Model. *Science* 249:1564-1566; 1990).

For life-threatening side effects, efforts have concentrated on altering the dose and schedules of the chemotherapeutic agent to reduce the side effects.  
20 Other options are becoming available, such as the use of colony stimulating factor (CSF), granulocyte-macrophage-CSF (GM-CSF) or epidermal growth factor (EGF) to increase the number of normal cells in various tissues before the start of chemotherapy (See Jimenez,  
25 J.J.; Yunis, A.A. Protection from 1- $\delta$ -D-Arabinofuranosylctosine-Induced Alopecia by Epidermal Growth Factor and Fibroblast Growth Factor in the Rat Model. *Cancer Research* 52:413-415; 1992). The mechanisms of protection by these factors, while not  
30 fully understood, are most likely associated with an increase in the number of normal critical target cells before treatment with cytotoxic agents.

There are few compounds which provide direct protection from injury caused by chemotherapy. One  
35 agent that has been reported to protect the kidney from injury caused by bolus infusions of cisplatin is S-2-(3-aminopropylamino) ethylphosphorothioic acid

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(WR2721). (See Glover, D.; Fox, K.R.; Weiler, C.; Kligerman, M.M.; Turrisi, A.; Glick, J.H. Clinical Trials of WR-2721 Prior to Alkylating Agent Chemotherapy and Radiotherapy. *Pharmacology and Therapeutics* 39:3; 1988). This compound appears to have limited usefulness and is effective only for large single doses of the chemotherapy agent, since WR-2721 itself given in large protective doses causes hypotension, nausea, and vomiting in humans. These toxicities increase with daily use and, as a result, have been found ineffective given over the days to weeks of typical chemotherapy regimens.

It would be desirable to provide effective protection from chemotherapy side effects. It would be desirable to provide such protection by a simple procedure which would assure compliance and not interfere with the beneficial therapeutic properties of the chemotherapy agents.

Prostaglandins are known for their protective properties associated with the gastric and intestinal mucosa. They are recognized for their protective properties with respect to ethanol-induced injury to the G.I. tract, NSAID induced injury to the G.I. tract and to radiation-induced injury. The mechanism associated with ethanol injury appears to be related to mucous secretion and/or bicarbonate secretion. There is no apparent causal link between prostaglandins and WR-2721 which compound has been shown to protect from cisplatin injury.

U.S. Patent No. 4,081,553 (to Robert), provides a method for the treatment of intestinal inflammatory disease caused by radiation exposure, as well as a method of treatment for Crohn's disease, inflammatory bowel disease, infectious enteritis, sprue and intestinally manifested allergic responses to foodstuffs by administering a cytoprotective prostaglandin to a patient suffering from one of these



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diseases. Such cytoprotective prostaglandins modify inflammatory infiltration into gastric or intestinal tissue and are administered after injury to the tissue.

U.S. Patent No. 4,097,603 (to Robert) discloses a  
5 method of protecting gastric tissue from gastric  
inflammatory disease and ulcerative (erosive) diseases  
by administration of prostaglandins, including  
prophylactic administration 30 minutes prior to  
administration of an inflammation causing agent. The  
10 type of injuries to gastric tissue discussed by Robert  
are injuries resulting from inflammation of such  
tissue. Robert mentions that suitable subjects for  
treatment by the disclosed methodology include patients  
exposed to noxious agents such as household cleaners  
15 and chemotherapeutic agents.

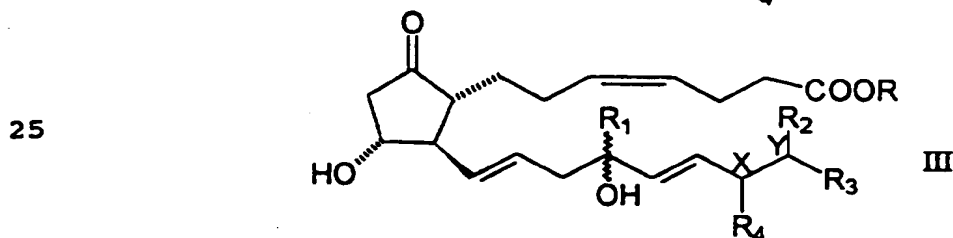
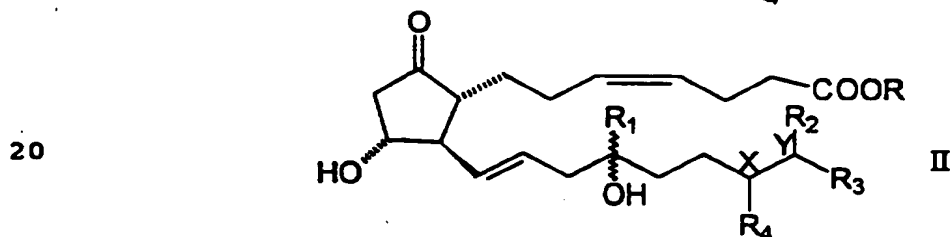
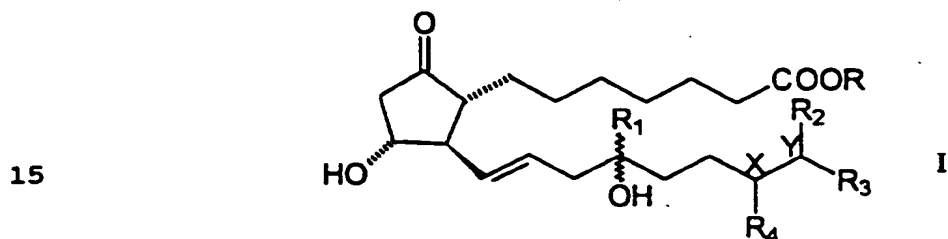
The mechanisms of protection by prostaglandins of  
gastric and intestinal tissue are associated with  
increased mucous secretion or increased bicarbonate  
secretion. (See T.A. Miller, Am. J. Physiol., 245:  
20 G601-G623, 1983). Such increased secretions are unique  
to the physiology of gastric and intestinal tissue and  
such mechanisms would not be possible in non-mucous  
and/or non-bicarbonate secreting tissue.

The present invention provides cell protection for  
25 several non-mucous producing and non-bicarbonate  
producing tissues, such as hair follicles, bone marrow  
and skin, from cell injury caused by chemotherapeutic  
agents. Additionally, the present invention provides  
for protection of normal tissue from actual cell death  
30 which is distinct from the protection of stomach and  
intestinal tissue from inflammatory diseases. Thus, it  
is postulated that the mechanism of action of the E-  
type prostaglandins of the present invention is unique  
and distinct from the proposed mechanism of action of  
35 PG induced protection of stomach tissue or intestinal  
tissue from inflammation.

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Summary of the Invention

The present invention provides a method for protecting non-stomach tissue in a mammal from injury resulting from chemotherapeutic agents comprising, administering, prior to the administration of such chemotherapeutic agent, a therapeutically effective amount of an E-type prostaglandin. More preferably the E-type prostaglandin administered is selected from the group consisting of:



30 16,16-dimethyl PGE<sub>2</sub>; and  
PGE<sub>1</sub>.

wherein R represents hydrogen or lower alkyl having 1 to 6 carbon atoms; R<sub>1</sub> represents hydrogen, vinyl or lower alkyl having 1 to 4 carbon atoms and the wavy line represents R or S stereochemistry; R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are hydrogen or lower alkyl having 1 to 4 carbon atoms or R<sub>2</sub> and R<sub>3</sub> together with carbon Y form a cycloalkenyl

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having 4 to 6 carbon atoms or  $R_3$  and  $R_4$  together with carbons X and Y form a cycloalkenyl having 4 to 6 carbons and wherein the X-Y bond can be saturated or unsaturated. Most preferably the E-type prostaglandin administered is misoprostol.

#### Brief Description of the Drawings

Figure 1 is a graph illustrating percent survival of mice over 15 days as a function of the time interval (in hours) between the administration of misoprostol and the subsequent administration of doxorubicin. (The two curves represent duplicate experiments; 12 animals per experiment.)

Figure 2 is a graph illustrating the percent survival of mice over 15 days following the administration of 0.5 mg of doxorubicin (open circles) and mice given 40  $\mu$ g of misoprostol 2 hours before administration of 0.5 mg of doxorubicin (filled circles). (N=16 for each group).

Figure 3 is a graph illustrating the percent survival of mice over 15 days following the administration of 0.75 mg of doxorubicin (open circles) and mice given 40  $\mu$ g of misoprostol 2 hours before administration of 0.75 mg of doxorubicin (filled circles). (N=16 for each group).

Figure 4 is a graph illustrating the percent survival of mice over 15 days following the administration of 12.5 mg of 5-fluorouracil (open circles) and mice given 40  $\mu$ g of misoprostol 2 hours before administration of 12.5 mg of 5-fluorouracil (filled circles). (N=8 for each group).

Figure 5 is a graph illustrating the percent survival of mice 15 days following administration of graded doses of doxorubicin (open circles) and mice given 40  $\mu$ g of misoprostol 2 hours before administration of graded doses of doxorubicin (filled circles). The open triangle is data of a group of 10

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mice given WR-2721, 30 minutes before administration of 0.75 mg of doxorubicin. (The lines are computer fit spline curves through the data; n=24 per data point on each curve).

5        Figure 6 is a graph illustrating the percent survival of mice over 15 days following administration of graded doses of doxorubicin (open circles) and of mice given 10  $\mu$ g of 16,16-dimethyl PGE<sub>2</sub> (open circles) or 25  $\mu$ g ( $\pm$ )methyl, 7-[3 $\alpha$ -hydroxy-2 $\beta$ -(4R-hydroxy-4-methyl-1E,5E,7E-nonatrienyl)-5-oxo-1 $\alpha$ -cyclopentyl]-4Z-heptenoate (open triangles) 2 hours before  
10       administration of graded doses of doxorubicin (filled circles). (The lines are computer fit spline curves through the data; n=8 per data point for each of the  
15       PG-treated groups and n=24 for the data points of mice treated only with doxorubicin).

      Figure 7 is a graph illustrating the protective effect of misoprostol (given 2 hours before doxorubicin) on WBC counts compared to the effect of  
20       doxorubicin alone on WBC counts as function of time (in days). (The numbers 1 and 2 are duplicate studies.)

      Figure 8 is a representation of low power (30x) photomicrographs of hair from a 4.42 sq mm area of rump skin in an untreated control animal (Panel A) and from  
25       an animal treated for 5 days with a 0.1 mg daily dose of doxorubicin (Panel B). Panel C is a representation of a photomicrograph of an animal given 40  $\mu$ g of  
      misoprostol, 2 hours before the 0.1 mg daily dose of doxorubicin given each day for 5 days. Average hair  
30       counts are presented in Figure 9.

      Figure 9 is a graph illustrating the number of hairs per 4.42 sq. mm of skin area in control mice, in mice that received 0.1 mg of doxorubicin daily for 5 days or in mice that received 40  $\mu$ g of misoprostol 2  
35       hours before each of the daily doses of doxorubicin given for 5 days. (Data are the means of 6 mice (2 fields/mouse)  $\pm$  1 SEM.)

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Figure 10 is a graph illustrating the number of hairs per 4.42 sq mm of skin area in control mice, in mice that received 1.0 mg of cytoxan daily for 5 days, and in mice that received 40  $\mu$ g of misoprostol 2 hours before each of the daily doses of cytoxan given for 5 days. (Data are the means of 6 mice (2 fields/mouse)  $\pm$  1 SEM.)

Figure 11 is a graph illustrating the number of hairs per 4.16 sq. mm of skin area in control mice, in mice that received 0.5 mg of doxorubicin or in mice that received 10  $\mu$ g of PGE<sub>1</sub> 2 hours before receiving 0.5 mg of doxorubicin. (Data are the means of 6 mice (2 field/mouse)  $\pm$  SEM.)

Figure 12 is a graph illustrating the number of microcolonies per intestinal circumference in mice given 0.5 mg of doxorubicin or in mice given 10  $\mu$ g of PGE<sub>1</sub> 2 hours before administration of 0.5 mg of doxorubicin. (Data are the means of 4 mice  $\pm$  1 SEM.)

Figure 13 is a representation of photographs of shafts of hair showing a single line of medullary cells in control mice (Panel A) and in mice 4 days after they were given a single dose of 0.3 mg of VP-16-213 (Panel B). Panel C in Figure 13 shows a representation of a photograph of medullary cells of mice 4 days after they were given misoprostol subcutaneously 2 hours before administration of VP-16-213 and Panel D shows a representation of a photograph of medullary cells of mice 4 days after they were given misoprostol topically 2 hours before administration of VP-16-213.

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#### Detailed Description

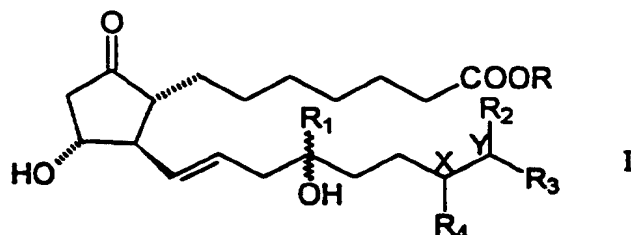
The present invention provides a method for protecting non-stomach tissue in a mammal from injury resulting from chemotherapeutic agents comprising, administering, prior to the administration of such chemotherapeutic agent, a therapeutically effective

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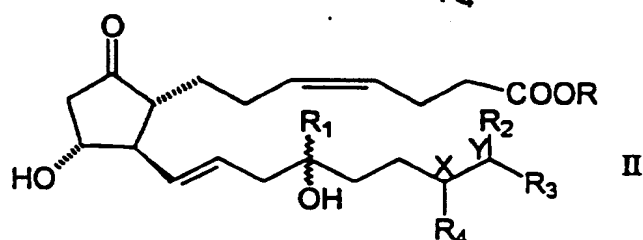
- 11 -

amount of an E-type prostaglandin. More preferably the E-type prostaglandin administered is selected from the group consisting of:

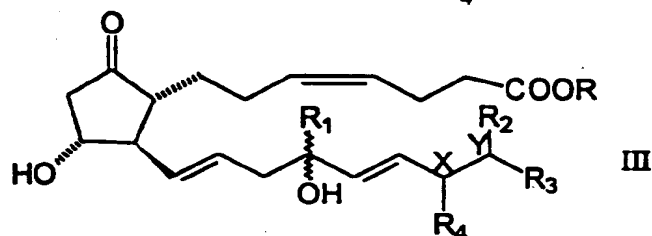
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16,16-dimethyl PGE<sub>2</sub>; and

PGE<sub>1</sub>,

25 wherein R represents hydrogen or lower alkyl having 1 to 6 carbon atoms; R<sub>1</sub> represents hydrogen, vinyl or lower alkyl having 1 to 4 carbon atoms and the wavy line represents R or S stereochemistry; R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are hydrogen or lower alkyl having 1 to 4 carbon atoms or R<sub>2</sub> and R<sub>3</sub> together with carbon Y form a cycloalkenyl having 4 to 6 carbon atoms or R<sub>3</sub> and R<sub>4</sub> together with carbons X and Y form a cycloalkenyl having 4 to 6 carbons and wherein the X-Y bond can be saturated or unsaturated. Most preferably the E-type prostaglandin administered is misoprostol.

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The invention further provides for the systemic or topical administration of such E-type prostaglandins,

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and more specifically provides for administration of the prostaglandins 1/2 hour to 3 hours prior to administration of the chemotherapeutic agent. The invention further provides for a method for inhibiting the reduction of lymphocytes in a mammal being treated with a lymphocyte-reducing chemotherapeutic agent, by administration of a therapeutically effective amount of the E-type prostaglandins described above. The invention provides protection for the cells of normal tissue from injury or cell death resulting from administration of chemotherapeutic agents.

As used herein the term "cell death" refers to the loss of reproductive integrity or clonogenic capacity resulting from the activity of the chemotherapeutic agent.

Protection of normal tissue of cancer patients from injury or cell death caused by systemic administration of chemotherapeutic agents may be accomplished by administering the prostaglandins herein, topically in an appropriate vehicle, to surface structures such as hair follicles, skin, oral and pharyngeal mucosa, esophagus, colon, rectum, bladder, vagina, or any organ on the outside of the body or internally where there is a luminal surface which can be reached non-invasively. Topical administration is especially suitable for protection of hair follicles and the oral mucosa. The protection of internal organs such as bone marrow, kidney, small intestine, liver, brain, gonads or any other normal internal organ susceptible to chemotherapy injury may be achieved by administration of a prostaglandin herein by any technique capable of introducing the compound into the blood stream of a patient, including oral administration and by intravenous, intramuscular and subcutaneous injections.

Prostaglandin compounds indicated for protection of normal tissue from injury or cell death

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by chemotherapeutic agents can be administered from about 30 minutes to about 3 hours before administration of a chemotherapeutic agent and more preferably the prostaglandins are administered about 1 hour to about 3 hours prior to administration of a chemotherapeutic agent. Most preferably the prostaglandin is administered from about 1.5 hours to about 2.5 hours before administration of a chemotherapeutic agent. While some protection may be provided by administering the prostaglandins outside of these ranges, greater protection is provided within this range.

The dosage of protective prostaglandins administered will depend upon patient condition and symptomology. The therapeutically effective amount of prostaglandin administered will be in an amount of about 0.010  $\mu\text{g}$  up to about 50  $\mu\text{g}$  per kilogram of body weight per day. A more preferred dosage range is from about 0.1  $\mu\text{g}$  to about 20  $\mu\text{g}$  per kilogram of body weight. The most preferred dose is in an amount from about 0.2  $\mu\text{g}$  to about 10.0  $\mu\text{g}$  per kilogram of body weight per day. A suitable dose can be administered in multiple sub-doses per day. These sub-doses can be administered in unit dosage forms. Typically, a dose or sub-dose may contain from about 5.0  $\mu\text{g}$  to about 800  $\mu\text{g}$  of active compound per unit dosage form. A more preferred dosage can contain from about 10  $\mu\text{g}$  to about 400  $\mu\text{g}$  of active compound per unit dosage form. The most preferred dosage form containing from about 10  $\mu\text{g}$  to about 200  $\mu\text{g}$  of active compound per unit dose.

The active compound can be administered in a pharmaceutically-acceptable formulation. Such formulations may comprise the active E-type prostaglandin together with one or more pharmaceutically-acceptable carriers or diluents. Such formulations may comprise more than one of the active E-type prostaglandins. Other therapeutic agents may also be present in the formulation. A



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pharmaceutically-acceptable carrier or diluent provides an appropriate vehicle for delivery of the active compound without introducing undesirable side effects. Delivery of the active compound in such formulations may be by various routes including oral, nasal, topical, buccal and sublingual, or by parenteral administration such as subcutaneous, intramuscular, intravenous and intradermal routes.

Formulations for oral administration may be in the form of capsules or tablets containing the active compound dispersed in a binder such as gelatin or hydroxypropylmethyl cellulose, together with one or more of a lubricant, preservative, surface-active agent or dispersing agent. Such capsules or tablets may contain a controlled-release formulation as may be provided by a dispersion of active compound in hydroxypropylmethyl cellulose.

Formulations for parenteral administration may be in the form of aqueous or nonaqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration.

The following examples describe and illustrate embodiments of the present invention. These examples are meant to be illustrative of the present invention and not limiting thereof in either scope or spirit.

### Biological Evaluation

The utility of the prostaglandins herein as protectors from damage to normal tissue caused by chemotherapeutic agents used to treat human cancer, is demonstrated by the following assays and results.

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EXAMPLE 1

Mortality of mice is a measure of PG-induced protection from death due to severe normal tissue injury inflicted by chemotherapy agents.

5        This assay was accomplished by injecting intraperitoneally (IP) separate groups of mice with a range of doses of chemotherapeutic agents. The dose range of each chemotherapeutic agent varied between low doses which caused no death to high doses that caused  
10       100 percent death within a specified time. In some groups of mice, longevity was recorded by plotting the number of animals remaining alive (percent survival) versus time following the administration of the chemotherapeutic agent.

15       PG-induced protection from the toxic chemotherapeutic agents was measured by subcutaneous (SC) injection of the PG at various times prior to or after the IP administration of the chemotherapeutic agent. Figure 1 shows the percent of mice that  
20       survived for 15 days as a function of the time interval (in hours) between administration of misoprostol and the chemotherapeutic agent, doxorubicin (DOX). The time interval was varied from 15 minutes to 5 hours. The two curves represent two separate experiments with  
25       20 mice in each experiment. The "0" time point is the percentage of mice that survived 15 days after administration of doxorubicin alone. The results show that the optimum time interval to achieve near maximum misoprostol-induced protection when given prior to  
30       administration of doxorubicin is from about 1 to about 3 hours.

Figure 2 shows percent survival of mice (n = 16) given 0.5 mg of doxorubicin (DOX) and a separate group of mice (n = 16) given 40 µg of misoprostol 2 hours  
35       before administration of 0.5 mg of doxorubicin. By 15 days after administration of DOX alone, about 25% of the mice still survived. Of the group of mice that

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received the misoprostol before DOX, 100% of the mice were still alive after 15 days. Statistical analysis using the Life Table algorithm in the 4.01 version of SPSS/PC + software (SPSS Inc., Chicago, IL) showed a p value of  $\leq 0.001$ .

Figure 3 shows data similar to that in Figure 2; however, one group of mice ( $n = 16$ ) was given 0.75 mg of DOX/mouse and the other group of mice ( $n = 16$ ) was given 40  $\mu$ g misoprostol 2 hours before administration of 0.75 mg of DOX. All of the mice were dead by six days following the I.P. administration of 0.75 mg of DOX alone. Of the group of mice given 40  $\mu$ g misoprostol 2 hours before 0.75 mg of DOX, about 85% were still alive by day 15. There was a statistically significant difference at the  $p \leq 0.001$  value for these data.

The toxicity of 12.5 mg of 5-fluorouracil alone to mice and the effectiveness of 40  $\mu$ g of misoprostol given 2 hours prior to administration of 5-fluorouracil to protect from death due to 5-fluorouracil is shown in Figure 4. The data indicates that misoprostol was effective in protecting mice from damaging effects of 5-fluorouracil. The life table analysis showed that with an  $n$  of 8, the  $p$  value was  $\leq .05$ .

It was observed that all acute deaths due to DOX treatment alone occurred before day 15 following DOX administration. Subsequently, the parameter of death within 15 days was used to investigate the DOX dose response administered IP alone or 2 hours after 40  $\mu$ g of misoprostol was given subcutaneously (sc). In this study, a dose of about 0.5 mg of DOX/average 30 gram mouse, killed 50% of the DOX treated mice (see Figure 5). In groups of mice given 40  $\mu$ g of misoprostol 2 hours before administration of graded doses of DOX, the dose required to kill 50% of the population of mice, was increased to about 1.0 mg of DOX/average 30 gram mouse.

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In order to provide protection, the PG had to be given before the chemotherapeutic agent.

Administration of the PG 5 minutes or 1 hour after administration of DOX afforded no protection to DOX treated mice (data not shown).

A comparison of PG-induced protection was made to that of protection by WR-2721; an agent reported to protect from some chemotherapeutic agents. WR-2721 did not protect mice (n = 10) from DOX treatment, (see Figure 5). Two other PGs representing different structural prostaglandins than misoprostol were also investigated for their effectiveness in protecting mice from mortality resulting from administration of graded doses of DOX. Ten  $\mu$ g of 16,16-dimethyl PGE<sub>2</sub> and 25  $\mu$ g of ( $\pm$ )methyl, 7-[3 $\alpha$ -hydroxy-2 $\beta$ -(4R-hydroxy-4-methyl-1E,5E,7E-nonatrienyl)-5-oxo-1 $\alpha$ -cyclopentyl]-4Z-heptenoate (also known as SC-44932) increased the DOX dose necessary to kill 50% of a population (n=8) of mice (see Figure 6). The degree of protection by these PGs was similar to that seen for misoprostol.

The mechanism by which PGs protect from chemotherapeutic agents is not known; however, evidence suggests that several tissues are protected. Death within 15 days of DOX treatment alone is most likely associated with bone marrow injury. The white blood cells (WBCs) in the peripheral circulation of DOX treated mice (0.5 mg DOX/30 gram mouse) were sharply reduced over 7 days following treatment (See Fig. 7). In mice given misoprostol (40  $\mu$ g/30 gram mouse) 2 hours before DOX administration, the WBC counts remained near control levels. Since the turnover of circulating WBC is rapid (6 hours for neutrophils and within 1 to 3 days for many lymphocytes) these data suggest that misoprostol protects the WBC precursors in bone marrow from DOX injury. Differential counts showed that the percentage of lymphocytes was reduced from about 85% in control mice to less than 10% in mice given 0.5 mg of

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DOX. DOX, therefore, appears to not only reduce the total WBC, but has a particularly marked effect on lymphocytes. Differential counts of peripheral blood showed that the percentage of lymphocytes was increased  
5 from less than 10% in mice given 0.5 mg of DOX to greater than 30% in mice given 40  $\mu$ g of misoprostol 2 hours before administration of 0.5 mg of DOX.

Another example showing that PGs protect a variety of tissues from injury from chemotherapeutic agents is  
10 shown in the next example.

#### EXAMPLE 2

Amount of Hair Loss as a Measure of PG-induced Protection from Alopecia caused by Chemotherapeutic  
15 Agents.

One of the side effects of chemotherapy in cancer patients is hair loss or alopecia. Studies were conducted to measure a possible protective effect of PGs from alopecia caused by chemotherapeutic agents.  
20 The chemotherapeutic agents used for these studies were DOX and cytoxan (CTX). The hairs on the rumps of mice (n = 4/group) were plucked to induce a growth or anagen cycle within hair follicles which continues for about 18 days. Ten days after plucking, 5 daily treatments  
25 of DOX (0.1 mg/average 30g mouse/day) or CTX (1.0 mg/average 30g mouse/day) were administered I.P. In separate groups of plucked mice, misoprostol was given S.C. 2 hours before each dose of DOX or CTX. Control groups (n = 4) received no treatment or misoprostol  
30 alone.

All mice were killed 2 weeks after the initiation of the treatments and the number of hairs in 4.42 sq. mm areas were counted and averaged (2 fields per sample). Panel A of Figure 8 shows a representative  
35 field of untreated control hair under low power light microscopy. Panel B of Figure 8 shows a representative field of damage to hair in mice given fractions of DOX

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for 5 days. Panel C of Figure 8 shows a representative field from mice given misoprostol 2 hours before each of the daily treatments of DOX. The appearance of the hair in misoprostol plus DOX treated mice (Panel C) is similar to the appearance of normal untreated hair (Panel A).

The hair counts from skin fields of control (open bar), DOX (striped bar) and misoprostol + DOX (filled bar) treated mice are shown in Figure 9. DOX alone reduced the number of hairs from  $248 \pm 5$  per 4.42 sq. mm in controls to  $144 \pm 3$ . Misoprostol given before DOX increased the hair count to  $213 \pm 7.8$ . Similar data were found for CTX and misoprostol + CTX treated mice, see Figure 10. The values were  $248 \pm 5.5$  for controls (open bar),  $143 \pm 5.5$  for CTX treated mice (striped bar), and  $222 \pm 5.5$  for misoprostol + CTX treated mice (filled bar). Evidence suggests that prostaglandin protection of tissues may be dependent upon the receptors present in the tissue. Since prostaglandin receptors are not evenly distributed, protection may not be evenly distributed.  $\text{PGE}_1$  is associated with the skin (hair follicles) but not with the gut. The ability of  $\text{PGE}_1$  to protect the skin (hair follicles) and the small intestine from cell injury caused by doxorubicin was tested. Figure 11 shows the number of hairs ( $237 \pm 5$ ) per 4.16 sq. mm in control mice. A single dose of 0.5 mg of doxorubicin reduced this count to  $43 \pm 8$ . Administration of 10  $\mu\text{g}$  of  $\text{PGE}_1$  prior to administration of doxorubicin provided protection as indicated by the increase in the number of hairs to  $111 \pm 12$ . In Figure 12, the number of intestinal colonies per circumference in DOX treated mice ( $6 \pm 0.3$ ) did not change when 10  $\mu\text{g}$  of  $\text{PGE}_1$  was given 2 hours before administration of doxorubicin. These results suggest that the E-type prostaglandin misoprostol, binds to receptors and protects a broad range of tissues whereas  $\text{PGE}_1$  binds to receptors and

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protects a narrower range of tissues and does not protect the cells of the intestine from doxorubicin. These data provide strong evidence that misoprostol and PGE<sub>1</sub> protect hair follicles from injury resulting from the administration of chemotherapeutic agents. More evidence is presented in Example 3.

### EXAMPLE 3

Number of Hair Medullary Cells as a Measure of Protection from Injury caused by Chemotherapeutic Agents.

The shafts of hair in CF<sub>1</sub> mice contain usually a single line of about  $50 \pm 1$  medullary cells. These cells are very sensitive to cytotoxic agents (see Potten, C.S., Geng, L., and Taylor, P., Hair Medullary Cell Counts: a Simple and Sensitive Indicator of Radiation Exposure. *Int. J Radiat. Biol.*, 57:13-21, 1990). Single doses of cytotoxic chemotherapeutic agents greatly reduce the number of medullary cells which reach a nadir at 4 days after administration of the agent. This cell system has been used for the evaluation of misoprostol-induced protection from several chemotherapeutic agents.

CF<sub>1</sub> mice had hair from an area of their rump plucked to induce anagen growth as described in Example 2 above. Two weeks later, mice were divided into groups of 4 each and given the following treatments: controls (no treatment), or mice that received single doses of one of the following chemotherapeutic agents: DOX, CTX, etoposide (VP-16-213) cytosine arabinoside (ARA-C), Hydroxyurea (HU), 5-Fluorouracil (5-FU), methotrexate, bleomycin, mitomycin C, cisplatin, carboplatin or taxol. In separate groups, mice received misoprostol (40  $\mu$ g/average 30g mouse) either subcutaneously or applied topically 2 hours before each of the chemotherapeutic agents listed above. Untreated controls were used since it has been previously

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determined that water, or 2% EtOH in water, or phosphate buffer at pH = 6.8 (the vehicles for the all agents used) had no influence on the cell counts compared to untreated controls. Twenty-five hairs from each animal of each group were dissected from Feulgen stained samples taken from the mice 4 days after treatment.

Figure 13 shows representations of photomicrographs of the medullary cells from the hairs of control mice (Panel A), mice that received VP-16-213 (Panel B), mice that received misoprostol sc before VP-16-213 (Panel C), and mice that received misoprostol topically before VP-16-213 (Panel D). The number of medullary cells was averaged ( $n = 4$ ) for each treatment group and the results for all chemotherapeutic agents tested to date are presented in Table I.



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Table I.			
The average number of hair medullary cells four days following treatment in mice given a single dose of several chemotherapeutic agents or in mice given misoprostol 2 hrs. before the chemotherapeutic agents			
Treatment	Number of Medullar Cells $\pm$ 1 SEM		
	Chemotherapeutic agent IP	Chemotherapeutic Agent IP	
		Misoprostol Subcutaneously	Misoprostol Topical
control (no treatment)	51.4 $\pm$ 0.6	--	--
DOX (0.3 mg)	18.3 $\pm$ 1.7	30.0 $\pm$ 1.1	27.0 $\pm$ 1.3
CTX (1 mg)	22.1 $\pm$ 1.9	33.1 $\pm$ 1.3	31.5 $\pm$ 2.2
VP-16-213 (0.3 mg)	23.9 $\pm$ 2.0	34.4 $\pm$ 1.1	33.0 $\pm$ 1.6
ARA-C (8 mg)	39.0 $\pm$ 1.4	40.1 $\pm$ 0.7	43.1 $\pm$ 1.7
HU (10 mg)	34.1 $\pm$ 2.1	38.9 $\pm$ 0.7	40.7 $\pm$ 1.0
5 FU (1.0 mg)	38.4 $\pm$ 4.1	37.4 $\pm$ 5.0	--
Methotrexate (0.2 mg)	38.2 $\pm$ 2.3	50.6 $\pm$ 0.6	46.7 $\pm$ 2.2
Bleomycin (0.05 mg)	30.4 $\pm$ 1.4	27.9 $\pm$ 1.2	28.1 $\pm$ 1.0
Mitomycin C (0.01 mg)	26.8 $\pm$ 4.5	38.2 $\pm$ 1.9	--
Cisplatin (0.25 mg)	25.9 $\pm$ 1.5	14.3 $\pm$ 0.4	--
Carboplatin (1.0 mg)	37.8 $\pm$ 2.4	31.2 $\pm$ 2.4	--
Taxol (0.6 mg)	28.0 $\pm$ 3.1	26.0 $\pm$ 3.5	--

- Misoprostol afforded significant ( $p \leq .01$ ) protection of medullary cells from injury caused by DOX, CTX, VP-16-213, methotrexate, mitomycin C, and for HU treated mice when the PG was administered topically.
- 5 Misoprostol did not protect medullary cells in ARA-C bleomycin, HU (misoprostol given systemically), cisplatin, carboplatin or 5-FU treated mice although the values for mice given 5-FU in all cases were

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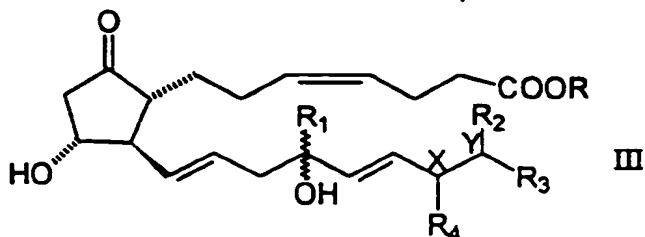
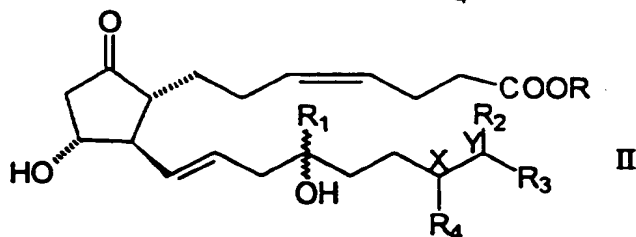
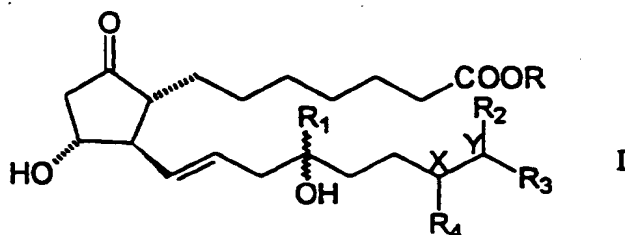
considerably lower than for the other chemotherapeutic agents.

The test results presented herein demonstrate that administration of misoprostol and/or other E-type  
5 prostaglandins, prior to administration of various chemotherapeutic agents provides protection of various normal tissues from injury or cell death caused by the chemotherapeutic agent. Although all of the E-type  
10 prostaglandins may not be effective in protecting all normal tissue from injury or cell death caused by every chemotherapeutic agent, one skilled in the art, in view of the present disclosure and methodology therein, would be able to determine which E-type prostaglandins are effective from protecting which tissues from injury  
15 or cell death caused by different chemotherapeutic agents. Furthermore from the results of the testing disclosed herein, it can be postulated that administration of combinations of more than one E-type prostaglandins would be at least as beneficial as  
20 administration of one such E-type prostaglandin in protecting normal tissue from injury and/or cell death depending on: 1) the presence of receptors within any given tissue for any E-type prostaglandin; 2) the types of receptors within a given tissue; and 3) the  
25 combination of chemotherapeutic agents. Such E-type prostaglandin induced normal tissue protection, as disclosed herein, would be beneficial to patients undergoing chemotherapy treatments, in that it could be expected that such tissue protection would result in  
30 reduced side-effects and increase the quality of life, and the longevity of such patients.

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What is claimed is:

1. A method for protecting non-stomach tissue in a mammal from injury resulting from chemotherapeutic agents comprising, administering prior to the administration of such chemotherapeutic agent, a therapeutically effective amount of an E-type prostaglandin.
2. A method as recited in Claim 1 for protecting tissue from injury resulting from chemotherapeutic agents comprising, administering prior to the administration of such chemotherapeutic agent, an E-type prostaglandin selected from the group consisting of:



16,16-dimethyl PGE<sub>2</sub>; and  
PGE<sub>1</sub>,

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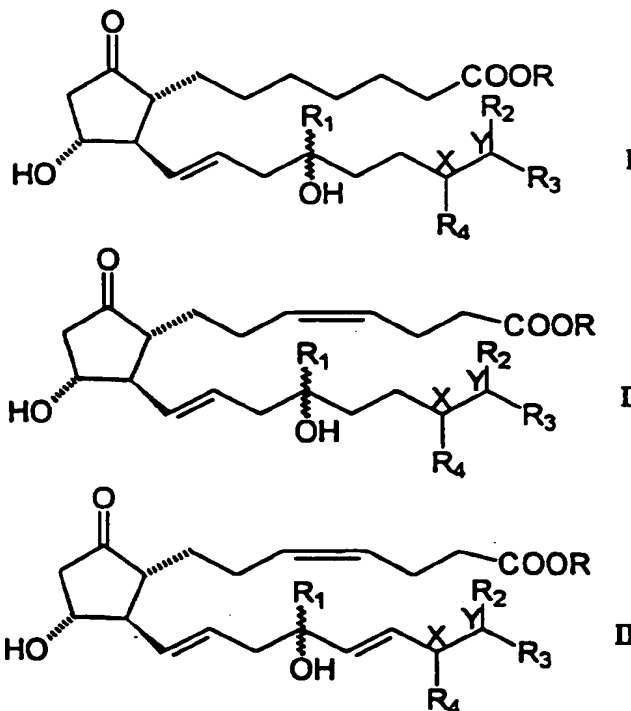
wherein R represents hydrogen or lower alkyl having 1 to 6 carbon atoms;  $R_1$  represents hydrogen, vinyl or lower alkyl having 1 to 4 carbon atoms and the wavy line represents R or S stereochemistry;  $R_2$ ,  $R_3$ , and  $R_4$  are hydrogen or lower alkyl having 1 to 4 carbon atoms or  $R_2$  and  $R_3$  together with carbon Y form a cycloalkenyl having 4 to 6 carbon atoms or  $R_3$  and  $R_4$  together with carbons X and Y form a cycloalkenyl having 4 to 6 carbons and wherein the X-Y bond can be saturated or unsaturated.

3. A method as recited in Claim 2 wherein the E-type prostaglandin is misoprostol.
4. A method as recited in Claim 3 wherein the E-type prostaglandin is administered in a dose of from about 0.01  $\mu\text{g/kg/day}$  to about 50  $\mu\text{g/kg/day}$ .
5. A method as recited in Claim 3 wherein the E-type prostaglandin is administered topically.
6. A method as recited in Claim 2 wherein the E-type prostaglandin is administered 1 to 3 hours prior to administration of the chemotherapeutic agent.
7. A method as recited in claim 2 wherein the E-type prostaglandin is administered topically.
8. A method as recited in Claim 2 wherein the E-type prostaglandin is administered 1 to 3 hours prior to administration of the chemotherapeutic agent.
9. A method as recited in Claim 2 wherein the E-type prostaglandin is ( $\pm$ )methyl 7-[3 $\alpha$ -hydroxy-2 $\beta$ -(4R-hydroxy-4-methyl-1E,5E,7E-nonatrienyl)-5-oxo-1 $\alpha$ -cyclopentyl]-4Z-heptenoate, or 16,16-dimethyl PGE<sub>2</sub>.

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10. A method as recited in Claim 9 wherein the E-type prostaglandin is administered in a dose of from about 0.01  $\mu\text{g/kg}$  to about 50  $\mu\text{g/kg/day}$ .
11. A method as recited in Claim 10 wherein the E-type prostaglandin is administered topically.
12. A method as recited in Claim 9 wherein the E-type prostaglandin is administered 1 to 3 hours prior to administration of the chemotherapeutic agent.
13. A method as recited in Claim 2 wherein the E-type prostaglandin is 16,16-dimethyl  $\text{PGE}_2$ .
14. A method as recited in Claim 13 wherein the E-type prostaglandin is administered in a dose of from about 0.01  $\mu\text{g/kg}$  to about 50  $\mu\text{g/kg/day}$ .
15. A method as recited in Claim 13 wherein the E-type prostaglandin is administered topically.
16. A method as recited in Claim 13 wherein the E-type prostaglandin is administered 1 to 3 hours prior to administration of the chemotherapeutic agent.
17. A method for inhibiting the reduction of lymphocytes in a mammal being treated with a lymphocyte-reducing chemotherapeutic agent by administering a therapeutically-effective amount of an E-type prostaglandin selected from the group consisting of:

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16,16-dimethyl PGE<sub>2</sub>; and

PGE<sub>1</sub>,

wherein R represents hydrogen or lower alkyl having 1 to 6 carbon atoms; R<sub>1</sub> represents hydrogen, vinyl or lower alkyl having 1 to 4 carbon atoms and the wavy line represents R or S stereochemistry; R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are hydrogen or lower alkyl having 1 to 4 carbon atoms or R<sub>2</sub> and R<sub>3</sub> together with carbon Y form a cycloalkenyl having 4 to 6 carbon atoms or R<sub>3</sub> and R<sub>4</sub> together with carbons X and Y form a cycloalkenyl having 4 to 6 carbons and wherein the X-Y bond can be saturated or unsaturated, prior to administration of the chemotherapeutic agent.

18. A method as recited in Claim 17 wherein the E-type prostaglandin is administered 1 to 3 hours prior to administration of the chemotherapeutic agent.

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19. A method of protecting cells of normal tissue in a mammal from cell death resulting from chemotherapeutic agents comprising, administering, prior to the administration of the chemotherapeutic agent, a therapeutically effective amount of an E-type prostaglandin.
20. The method of Claim 19 wherein the E-type prostaglandin is administered from about 1 to about 3 hours prior to administration of the chemotherapeutic agent.
21. A method of preventing alopecia induced by chemotherapeutic agents in a mammal, comprising administering a therapeutically effective amount of an E-type prostaglandin prior to administration of the chemotherapeutic agent.
22. A method as recited in claim 21 wherein the E-type prostaglandin is misoprostol.
23. A method as recited in Claim 21 wherein the E-type prostaglandin is PGE<sub>1</sub>.
24. A method for protecting tissue in a mammal from injury resulting from chemotherapeutic agents comprising, administering prior to the administration of such chemotherapeutic agent, a therapeutically effective amount of two or more E-type prostaglandins.
25. A method as recited in claim 24 wherein the E-type prostaglandins administered are misoprostol and PGE<sub>1</sub>.

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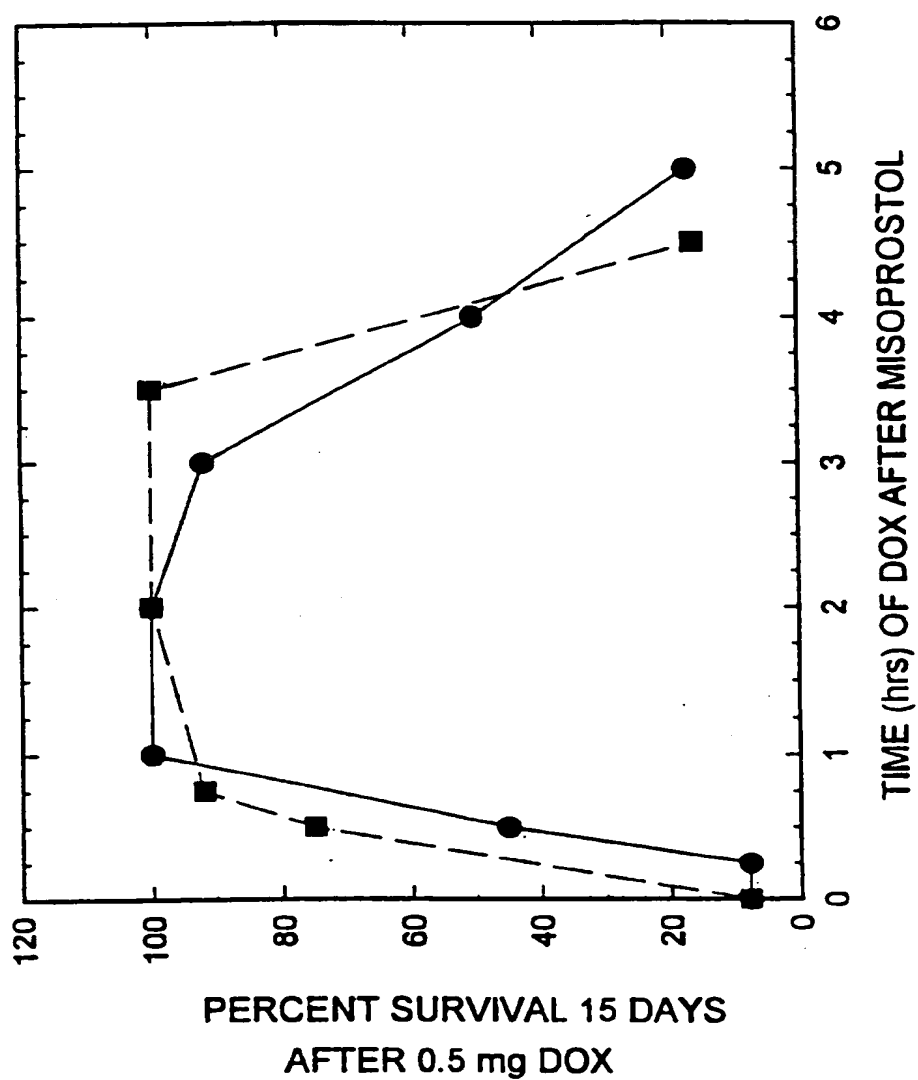
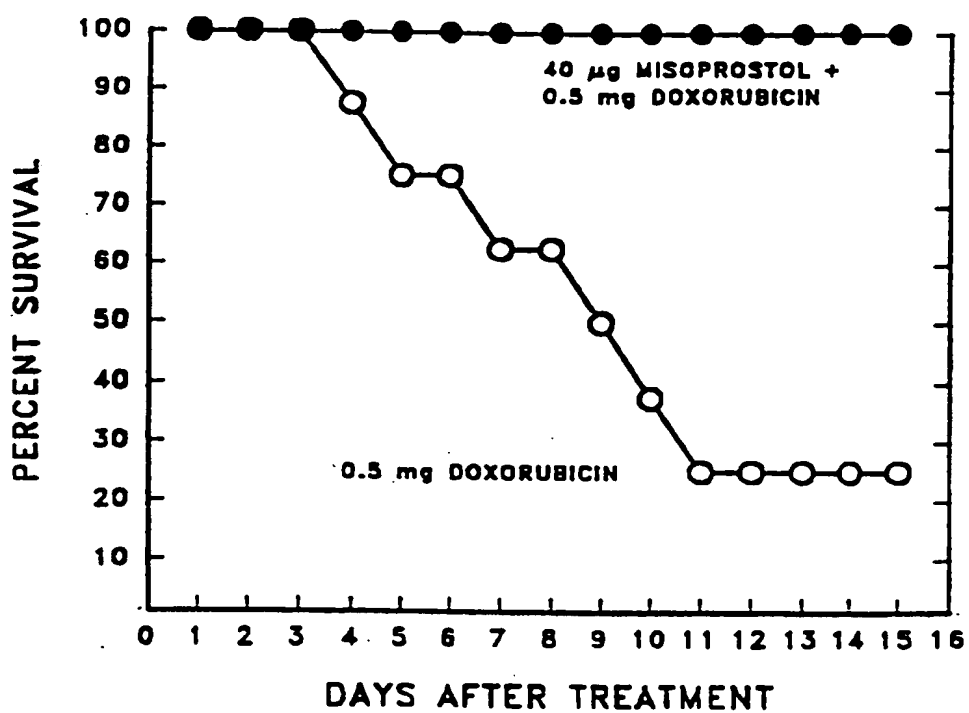


FIG. 1



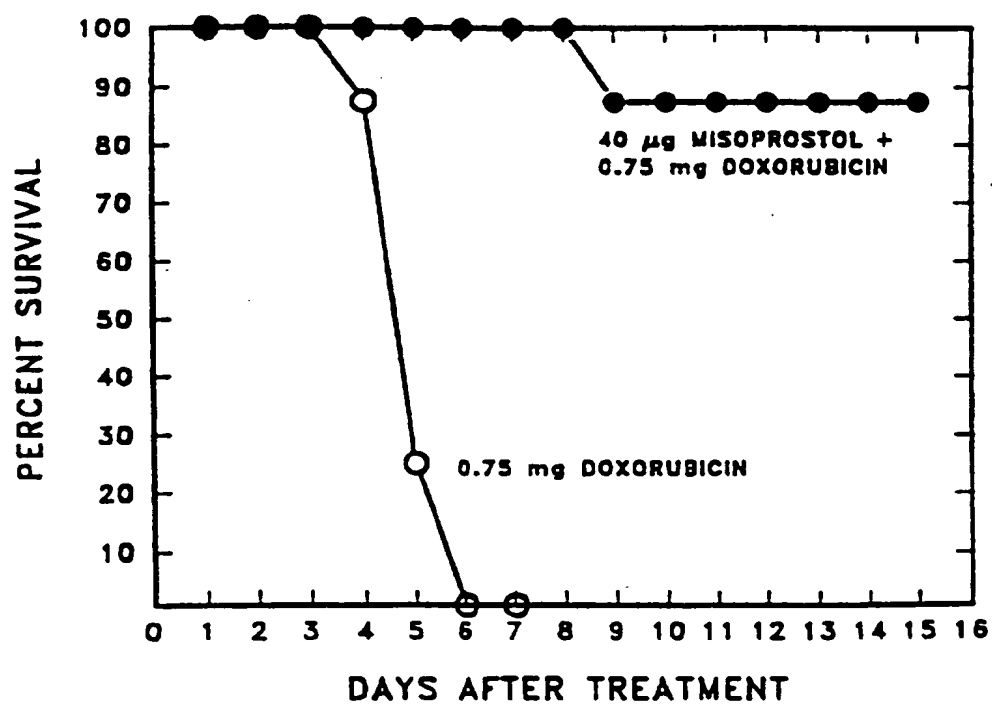
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FIG. 2



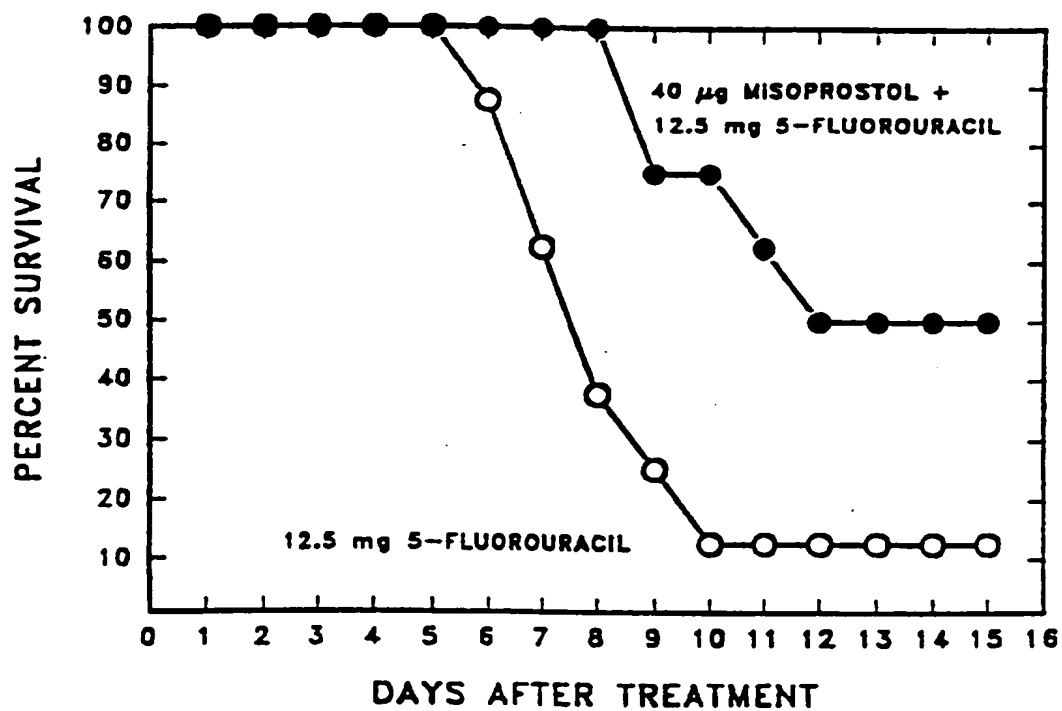
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FIG. 3



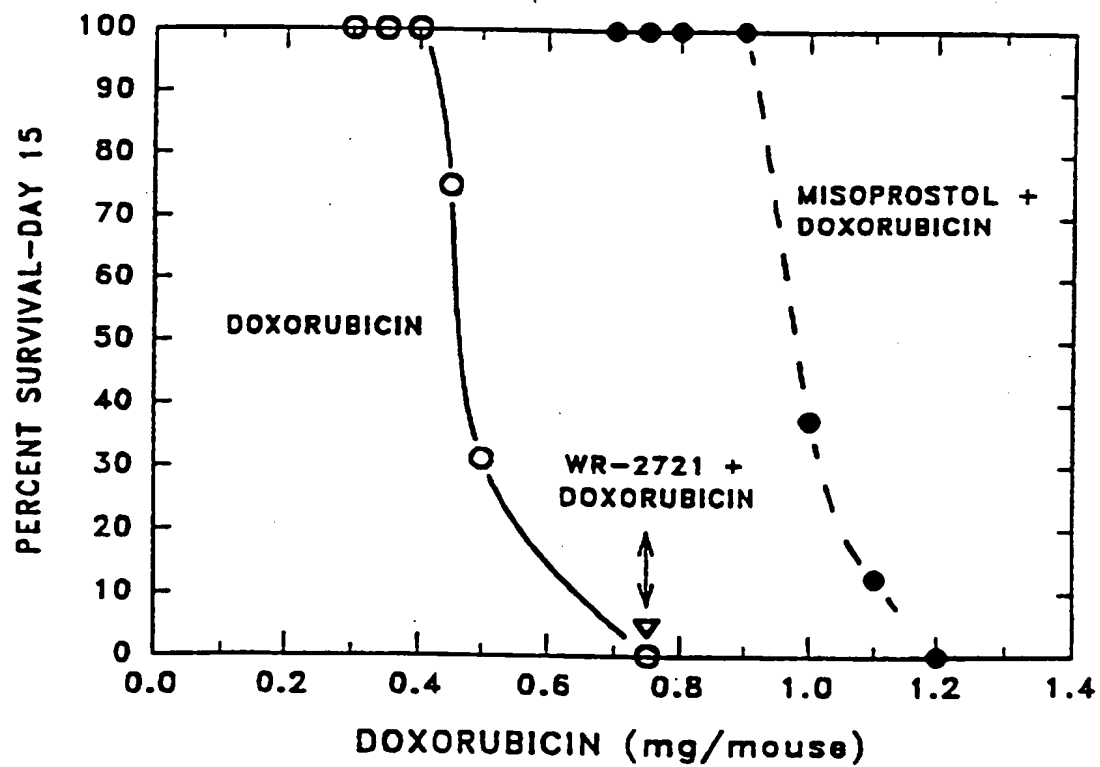
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FIG. 4



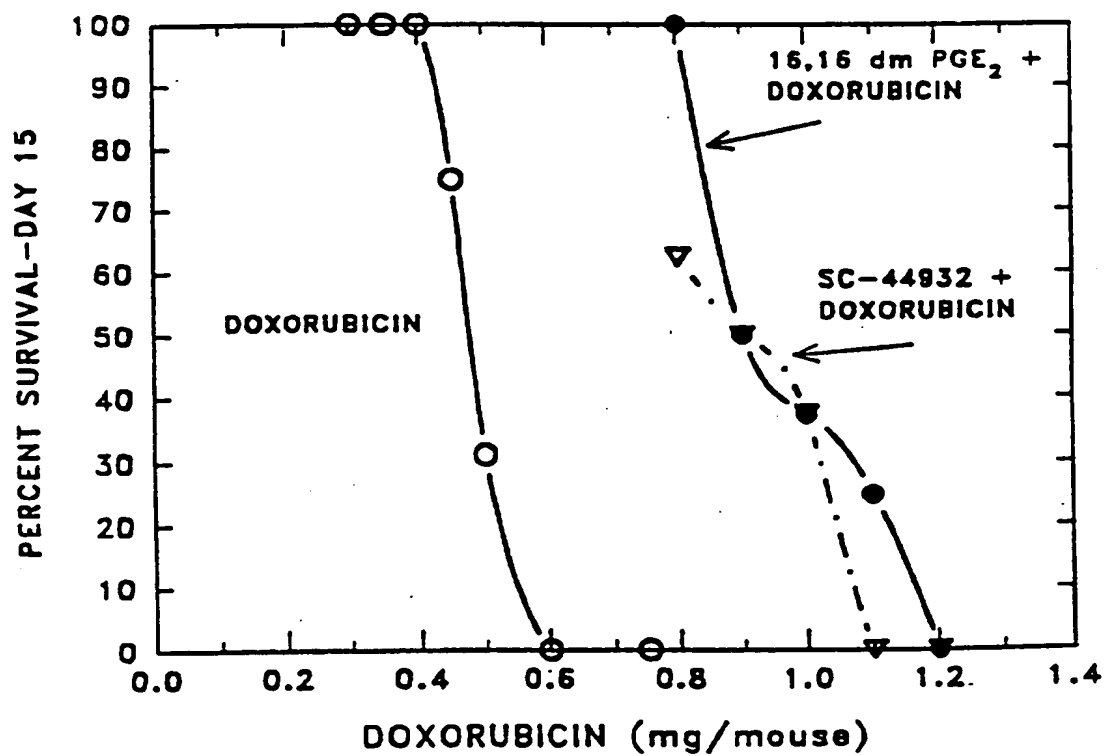
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FIG. 5



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FIG. 6



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FIG. 7

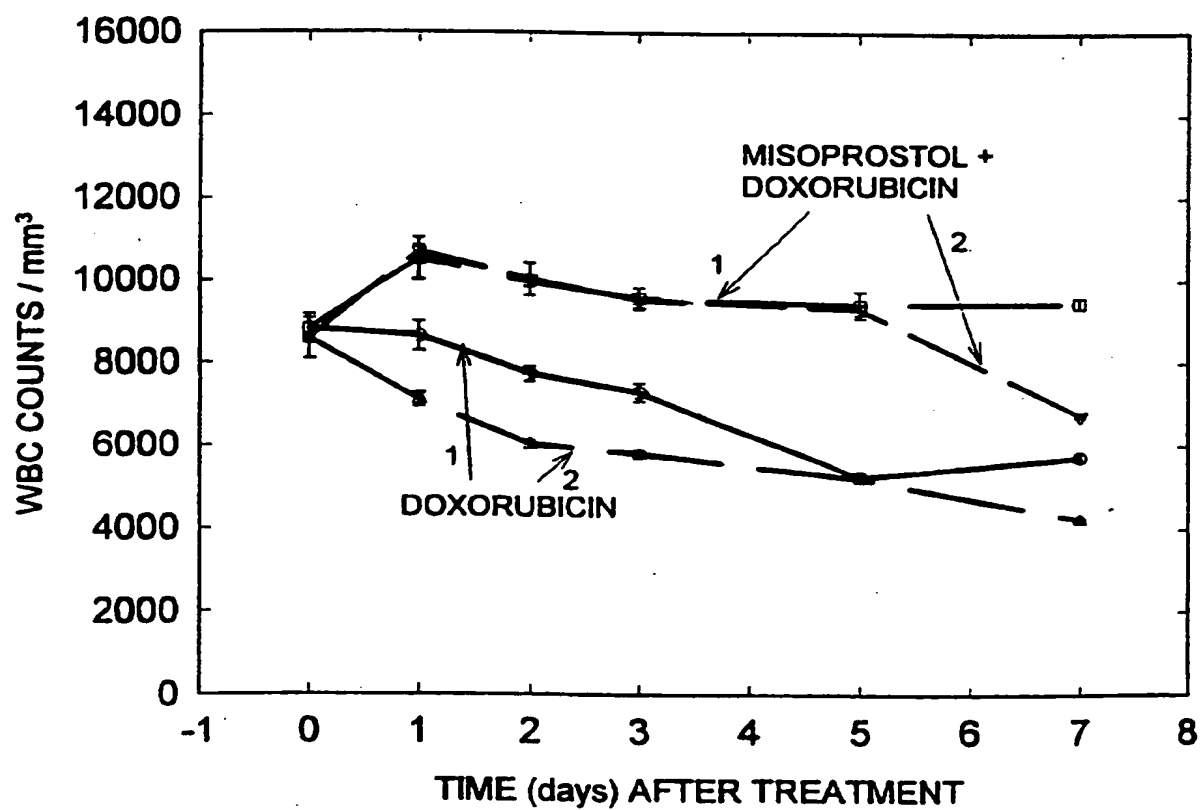




FIG. 8C

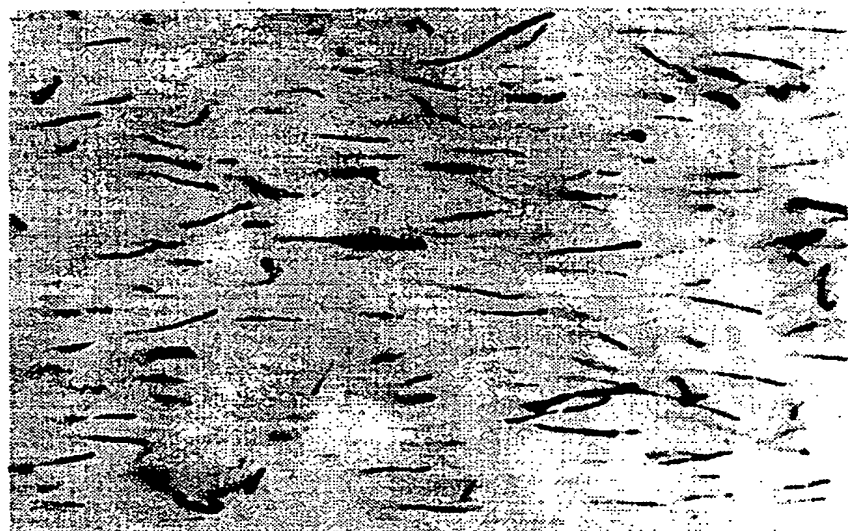


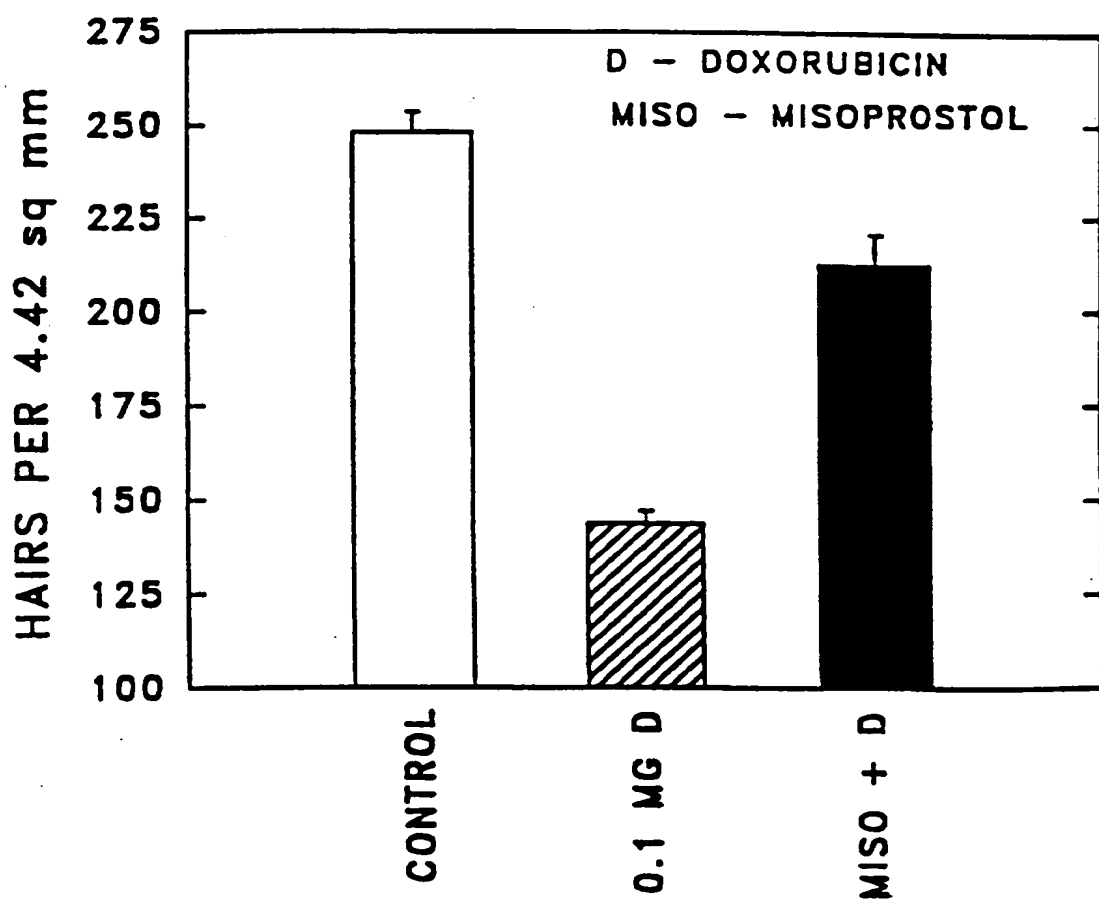
FIG. 8B



FIG. 8A

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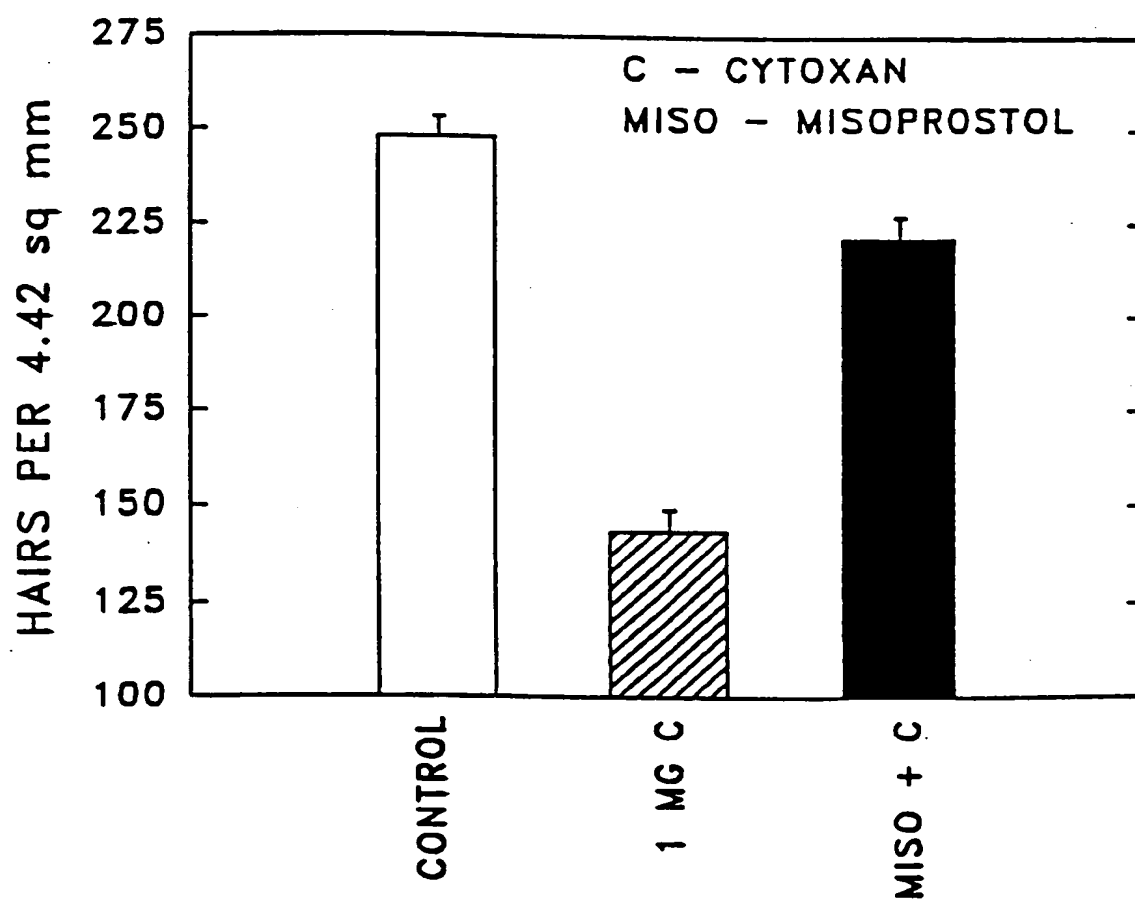
FIG. 9





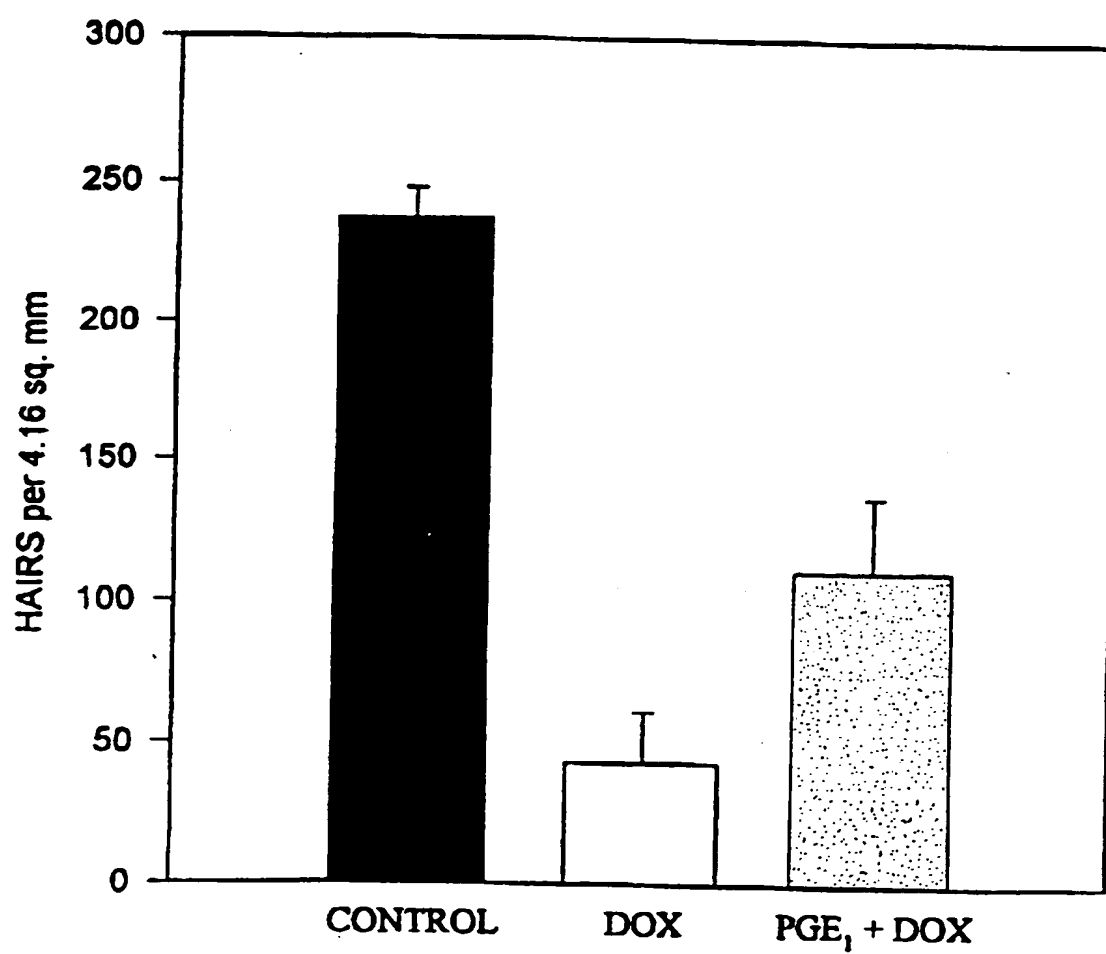
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FIG. 10



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FIG. II



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FIG. 12

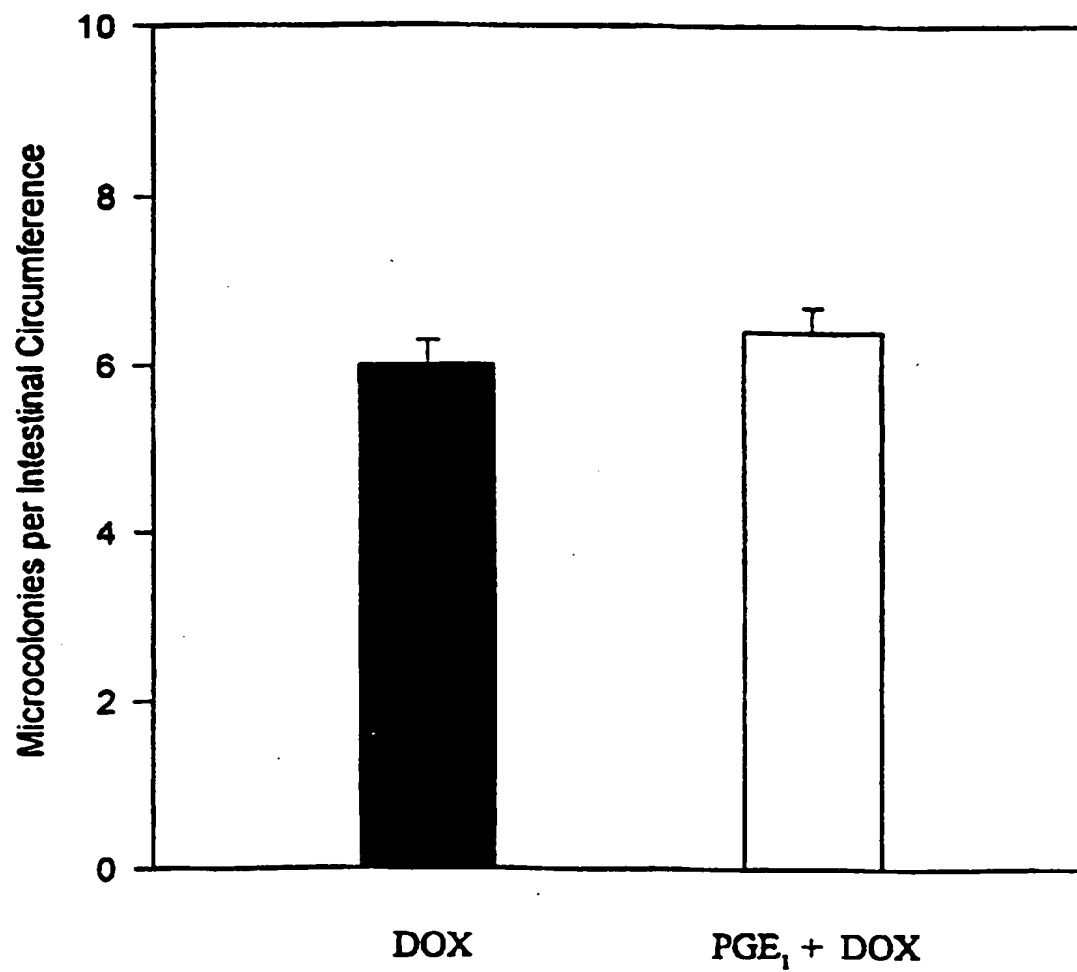




FIG. 13A



FIG. 13B

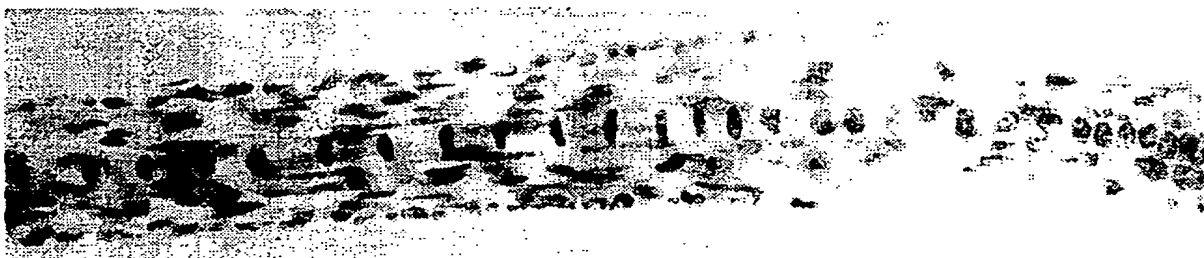


FIG. 13C

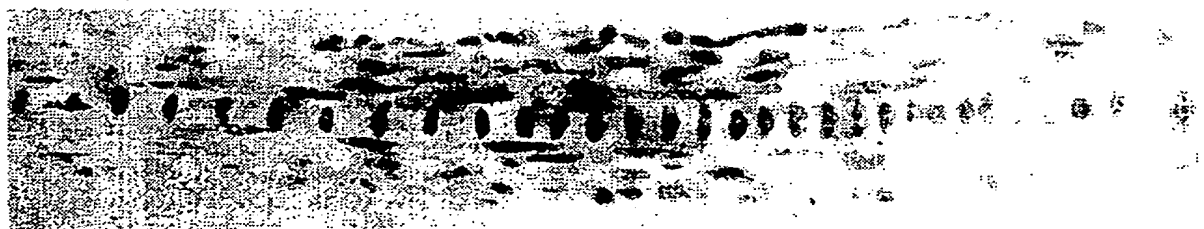


FIG. 13D

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/09677

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/557

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE,A,37 05 092 (BAYER AG) 1 September 1988	1-25
Y	see the whole document	1-25
Y	WO,A,94 12186 (SCHERING AG ;ZAFFARONI NADIA (IT); VILLA RAFFAELLA (IT); ORLANDI L) 9 June 1994	1-25
P,Y	see the whole document	
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	RADIATION RESEARCH, vol. 142, no. 3, 30 June 1995 pages 281-287, W.R. HANSEN ET AL 'The Prostaglandin E1 Analog, Misoprostol, a Normal Tissue Protector, Does Not Protect Four Murine Tumors In Vivo from Radiation Injury' see abstract	1-25
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☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

22 December 1995

Date of mailing of the international search report

16.01.96

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Authorized officer

Herrera, S

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/09677

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-3705092	01-09-88	NONE	
WO-A-9412186	09-06-94	NONE	